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Plasma DHA Is Related to Sleep Timing and Duration in a Cohort of Mexican Adolescents

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ABSTRACT

Background: Delayed sleep timing and short sleep duration represent a significant public health burden in adolescents. Whether intake of nutrients affects the pineal gland, where sleep/wake cycles are regulated, remains unclear.

Objectives: In a cross-sectional analysis, we investigated whether plasma concentrations of DHA and arachidonic acid (AA), long-chain fatty acids that can be obtained through diet, were related to sleep timing and duration in adolescents.

Methods: The study population included 405 Mexico City adolescents (mean age ± SD = 14.2 ± 2.1 y; 48% males) who took part in a 2015–2016 follow-up visit as a part of an ongoing cohort study. Fatty acid concentrations were measured in plasma using GLC, as a percentage of total fatty acids. Sleep midpoint and duration were assessed with 7-d wrist actigraphy. We categorized DHA and AA plasma concentrations into quartiles (Q1–Q4; Q4 = highest fatty acids). We conducted cross-sectional linear regression analysis with sleep characteristics as separate outcomes and quartiles of DHA and AA as exposures, adjusting for sex, age, and BMI *z*-scores.

Results: Mean ± SD plasma DHA (as percentage of total fatty acids) was 1.2 ± 0.4%, whereas mean ± SD plasma AA was 6.2 ± 1.5%. In adjusted analysis, higher plasma DHA was linearly associated with longer sleep duration on the weekends; to illustrate, those in Q4 compared with Q1 had 32 min longer duration (95% CI: 7, 57; *P* trend = 0.005). Higher DHA was also associated with earlier sleep timing during weekdays and weekends, although in a nonlinear fashion. The largest difference was a 0.75-h (45-min) later sleep midpoint in Q2 compared with Q4 (95% CI: 0.36, 1.14).

Conclusions: Plasma DHA was associated with earlier sleep timing and longer weekend sleep duration in Mexican adolescents. Whether DHA supplementation improves sleep in adolescent populations deserves consideration in randomized trials. *J Nutr* 2020;150:592–598.

Keywords: docosahexaenoic acid, arachidonic acid, sleep quality, actigraphy, Mexico

Introduction

Mounting evidence shows that insufficient sleep duration, poor sleep quality, and delayed sleep timing are associated with metabolic disturbance and obesity in adolescent populations (1, 2). The causes of poor sleep health in adolescence are multiple and include natural circadian delays (shifts later) in sleep timing, social media and screen use, early school start times, and lack of bedtime routines (3). Intake of particular foods or dietary patterns are another set of modifiable factors that could affect the duration and quality of sleep (4), yet have not received as much attention, particularly in adolescents.

In animal studies, long-chain fatty acid intake has been consistently linked with the regulation of sleep/wake cycles (5–7). The association has biological plausibility because the long-chain fatty acids docosahexaenoic acid (DHA) and arachidonic acid (AA) are known to play primary roles in the pineal gland, where melatonin—the hormone modulating sleep/wake cycles—is produced (8). Mice that have been deprived of DHA, or have a lower ratio of DHA:AA in the brain, have irregular melatonin production and dysregulated sleep/wake cycles (5–7). A few epidemiological studies in adults found that fatty fish intake (a source of DHA), concentrations of DHA, and/or ratio of DHA:AA in blood, were associated with higher sleep quality (9–11). Studies in infants have shown that maternal DHA supplement intake was positively associated with better sleep quality in the infants (12–14). Three relevant studies have been conducted in children (15–17). A randomized controlled trial (RCT) of DHA supplementation in boys with attention deficit/hyperactivity disorder (ADHD) reported that self-reported sleep quality improved in the experimental group compared with placebo (17). Another RCT in UK children underperforming in school reported that those supplemented with DHA had a 1-h longer sleep duration than the placebo group at follow-up, but only in a small subset of 43 children

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with objectively measured sleep (15). In contrast, an RCT in 4–6-y-old Norwegian children reported no difference in parent-reported sleep measures in the intervention group who were given fatty fish (herring and mackerel) for lunch 3 times per week compared with the control group who consumed meat (chicken, lamb, or beef) (16).

Given the scant and mixed evidence, our overall objective for the current study was to evaluate the relations between plasma DHA and AA with actigraphy-assessed sleep in a cohort of adolescents from Mexico City. Our central hypothesis was that DHA would be associated with longer sleep duration, early sleep timing, and lower sleep fragmentation (a measure of sleep quality). A secondary aim was to investigate the associations between AA and the ratio of DHA:AA with these sleep parameters.

Methods

Study population

The study sample included adolescent participants from 2 of 3 sequentially enrolled cohorts of the Early Life Exposure in Mexico to ENvironmental Toxicants (ELEMENT) study (18, 19). Between 1997 and 2004, 1012 mother/child dyads were recruited from prenatal clinics of the Mexican Social Security Institute in Mexico City, which serves low- to middle-income populations formally employed in the private sector. At baseline, mothers reported information on important sociodemographic and health characteristics. Children were followed approximately every 6–12 mo to 5 y of age and then periodically over mid-childhood and adolescence to gain information on growth and relevant environmental exposures. In 2015, a subset of 550 participants from the original birth cohorts 2 and 3, who were in the midst of pubertal transition (ages 9 to 17 y), were selected to participate in a follow-up visit that included blood collection and anthropometric assessment as well as 7 consecutive days of wrist actigraphy. The present analysis comprises a subset of 405 children who had actigraphy-assessed sleep as well as plasma measures of long-chain fatty acids. The institutional review boards at the Mexico National Institute of Public Health and the University of Michigan approved the research protocols. Informed consent was obtained from parents for all participants in addition to participant assent.

Plasma long-chain fatty acids

Adolescents provided a fasting blood sample, which was immediately separated and frozen at −80°C. Plasma samples were shipped for storage at −80°C at the University of Michigan School of Public Health. Of the sample of 528 participants with wrist actigraphy data, 405 participants were chosen at random for plasma fatty acid testing. Plasma fatty acid testing was conducted at the University of Michigan Metabolomics Core, using a standard protocol (20, 21). Briefly, total lipids were extracted from 100 μL of plasma according to the Bligh and Dyer method (20), with 10 μL of 4 mM nonadecanoic acid (19:0) added as an internal standard. Then the fatty acid components of the total lipids were derivatized into methyl esters using boron trifluoride-methanol. The methyl esters were extracted with a 2:1 hexane-water mixture and centrifugation (1000 × *g* for 30 min at 4°C), and then the hexane layer was transferred and dried. Next, the methyl esters were resuspended in 100 μL of hexane, and an auto-sampler injected 1–2 μL of sample into a gas chromatograph (Model 6890N; Agilent), which had a flame ionization detector and a 100 m × 0.25 mm × 0.2 mm SP-2560 column (Sigma-Aldrich). The temperature program was as follows: 100 to 180°C at 10°C/min, 180°C for 5 min, 180 to 190°C at 1°C/min, 190 to 210°C at 5°C/min, 210 to 250°C at 5°C/min, and 250°C for 10 min. Using Chemstation software version B.04 (Agilent), fatty acids were quantified in mM (amounts in micromoles)using a calibration curve prepared from the internal standard (C:19) and other authentic methyl esters. A total of 35 fatty acids were analyzed, and DHA and AA reported as a percentage of total mM fatty acids. Refer to **Supplemental Table 1** for mean ± SD of each of the fatty acids measured (as a percentage of total fatty acids in mM). Plasma DHA is considered a good proxy for DHA obtained through diet, whereas AA must be interpreted cautiously as a dietary proxy (21–24).

Sleep measures

At the end of the follow-up visit where blood was collected and dietary information gathered, adolescents were given an actigraph (ActiGraph GT3X+; ActiGraph LLC) to wear on their nondominant wrist continuously for 7 d. Nightly sleep parameters were estimated from the actigraphic data with the use of a fused lasso (least absolute shrinkage and selection operator)-based calculator package developed in R (R Foundation for Statistical Computing). As primary end points of the study, we obtained weekday and weekend sleep duration (minutes), weekday and weekend sleep midpoint (the median of sleep onset and wake time; reported in decimal hours), and mean sleep fragmentation index. Sleep fragmentation index was calculated as the percentage of 1-min (or shorter) periods of sleep out of the total number of sleep bouts of any length (25), with higher values representing more fragmented sleep. For sleep duration and midpoint, we stratified analyses by weekdays compared with weekends because the weekend days are typically not constrained by school/work schedules, allowing individuals to follow more natural sleep patterns in line with their internal body clock and external cues (e.g., light, food intake, physical activity).

Covariates

Possible confounders included in the present analysis were sex, age, BMI-for-age *z*-scores, maternal education, physical activity, screen time, smoking status, and caffeine consumption. Trained research assistants measured height in centimeters (BAME Model 420; Catalogo Medico) and weight in kilograms (BAME Model 420; Catalogo Medico). BMI *z*-scores accounting for age and sex were calculated based on the WHO reference (26). Maternal education was reported by mothers at the original cohort enrollment visit. Maternal education was categorized as <10 y, 10 to <12 y, 12 y, or >12 y. Physical activity (self-reported mean number of hours of moderate or vigorous activity per week, categorized in quartiles) and screen time (mean self-reported number of hours per week spent watching television, movies/DVD, or playing video games; categorized in quartiles) were assessed with a questionnaire adapted for and validated in Mexican adolescents (27). Smoking behavior was classified as a dichotomous variable to indicate whether the participants had ever tried smoking. Participants completed a 116-item semiquantitative FFQ based on the one used in the National Health and Nutrition Survey (ENSANUT-2012) and validated in the

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Data described in the manuscript, code book, and analytic code will be made available upon reasonable request.
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Abbreviations used: AA, arachidonic acid; ADHD, attention deficit/hyperactivity disorder; RCT, randomized controlled trial.

Mexican population (28). The data collected included the number of days, times per day, serving size, and number of servings consumed of each item listed, during the 7 d prior to the interview. To process the dietary information, the quantity of each food and drink was obtained by multiplying the number of days by the times per day, by the portion size (grams), and by the number of portions or pieces consumed on each occasion. Total grams were divided by 7 d to obtain the mean daily intake. For each food consumed, energy and macronutrients were calculated using a nutritional composition database of foods compiled by the National Institute of Public Health (29). The dietary variables we used in this analysis included total energy intake (kilocalories), caffeine (milligrams), and tuna/sardine intake (grams). Total energy intake and caffeine intake were conceptualized as potential confounders, whereas tuna/sardine intake was used as a marker of internal validity, to confirm there was a high correlation between plasma DHA and self-reported intake of tuna/sardine, which are among the main sources of DHA in this population (30). The dietary questionnaire also asked about usual intake of supplements or vitamins. In supplemental analyses, chicken and egg intake (grams per day) were also assessed.

Statistical analysis

First, associations between covariates and fatty acid concentrations were examined by comparing the mean ± SD of DHA and AA (as a percentage of total fatty acids) across categories of covariates. Spearman correlations of plasma DHA and AA were also run. Next, bivariate associations between fatty acids and sleep measures were evaluated by estimating the means ± SD of sleep duration (separately for weekday compared with weekend), midpoint (separately for weekday compared with weekend), and fragmentation index according to quartiles of DHA and AA. *P* values for trend (*P* trend) were obtained from Wald tests from linear regression models with sleep measures as the outcome and a variable representing quartiles of either DHA or AA. In the first set of complete-case multivariable analysis, separate linear regression models were run with continuous sleep measures (sleep duration, midpoint, and fragmentation index) as the outcome and indicator variables for quartiles of fatty acids as the exposure, adjusting for sex, age, maternal education, and BMI *z*-scores. Adjustment for the other potential confounders including an additional indicator of socioeconomic status did not alter estimates and thus were not retained. Fully adjusted models included DHA and AA in the same multivariable model, in addition to sex, age, maternal education, BMI *z*-scores, caffeine intake, and total energy intake. All analyses were conducted in Stata 14.0 (StataCorp LLC).

Results

Of the 550 adolescents who took part in the peripubertal study visit, 528 had wrist actigraphy sleep data; of these, a randomly selected subset of 405 adolescents had fatty acid concentrations assessed and were included in the present analysis (**Supplemental Figure 1**). The analytic sample was slightly younger than the adolescents not included, but did not differ with respect to sex, BMI *z*-scores, or maternal education. The mean ± SD age of the study sample was 14.2 ± 2.1 y, and 48% were male. The mean weekday sleep duration, midpoint, and fragmentation index were 504 ± 74 min, 3:83 ± 1.38 h, and 11.7 ± 4.2, respectively. Compared with weekdays, the mean weekend sleep duration was longer (mean = 544 ± 76 min), with later bedtimes (23:38 ± 1.3 h compared with 00:13 ± 1.5 h) and wake times (8:01 ± 1.7 h compared with 9:18 ± 1.3 h), whereas fragmentation index was not substantially different.

Mean plasma DHA was 1.2 ± 0.4% of total fatty acids, mean plasma AA was 6.2 ± 1.5%, and the mean ratio of DHA:AA was 0.20 ± 0.13. Mean estimated DHA intake based on the FFQ was 24 ± 24 mg; and the estimated combined DHA/EPA intake was 39 ± 37 mg. Plasma DHA and AA were positively correlated (Spearman correlation = 0.57; $P < 0.0001$). Associations between plasma fatty acids and sociodemographic and lifestyle characteristics are shown in **Table 1**. Higher plasma DHA was associated with female sex, lower BMI *z*-scores, and higher total grams of canned tuna and sardine intake. Compared with quartile 1 of plasma DHA, those in quartile 4 had 14.4 g/d more tuna/sardines (95% CI: 6.7, 22.1; ~2 servings/wk more). No adolescents reported intake of fish oil supplements. Neither chicken nor egg intake was linearly related to plasma DHA. Higher AA was only related to lower BMI-for-age *z*-scores. A higher ratio of DHA:AA was associated with female sex and higher tuna/sardine intake over the previous 7 d.

Neither DHA nor AA were associated with weekday sleep duration in separate models or in mutually adjusted models (models that included both DHA and AA; **Table 2**, all *P* trend > 0.05). However, lower plasma DHA was associated with shorter weekend sleep duration in both separate and mutually adjusted analyses (**Supplemental Table 2** and **Table 3**). To illustrate, participants in the 1st quartile had 32 min less sleep on the weekend compared with participants in the highest plasma fatty acid quartile (95% CI: 7, 57; *P* trend = 0.005).

In separate linear regression analyses, DHA and AA were each inversely associated with weekday sleep midpoint (Table 2). In mutually adjusted analyses (DHA and AA in same model), the associations were each attenuated and no longer had statistically significant dose–response relations. Nonetheless, a nonlinear statistically significant association remained for DHA. Compared with those in the 4th quartile of DHA, children in the 2nd quartile had a 0.75-h later weekday sleep midpoint (95% CI: 0.36, 1.14) after accounting for sex, age, BMI *z*-score, AA, caffeine, and total energy intake. The association was similar for weekend midpoint (Supplemental Table 1).

Neither DHA nor AA were statistically significantly associated with sleep fragmentation. The ratio of DHA:AA was not associated with any of the sleep measures. In addition, neither self-reported tuna/sardine intake nor estimated dietary DHA were statistically significantly related to any of the sleep outcomes, although the directions of the associations were consistent with the plasma DHA results (data not shown).

In sensitivity analyses that excluded children whose sleep was measured during summer months, estimates were slightly attenuated but not substantially altered; for example, the difference between 2nd and 4th quartiles for sleep midpoint was 0.60 h (95% CI: 0.17, 1.03 h; $P = 0.006$), and the difference between the 1st and 4th quartiles for weekend sleep duration was 30 min (95% CI: −58, −2 min; $P = 0.04$).

Discussion

In this cohort of Mexican adolescents, we found that plasma concentrations of DHA were related to sleep timing and duration. Adolescents with lower concentrations of plasma DHA (specifically, those in the 2nd quartile) had an approximately 45-min later sleep midpoint compared with the adolescents with the highest concentrations of DHA. Further, although DHA was not associated with sleep duration during weekdays, it was associated with a 30-min longer sleep duration on weekends, when sleep time is less likely to be restricted by school or work obligations. Given that even a

TABLE 1 Plasma fatty acid concentrations of 405 youth aged 9–17 y from Mexico City, according to sociodemographic and lifestyle characteristics[1]

Sociodemographic and lifestyle characteristics	*n*	DHA, % of total fatty acid	AA, % of total fatty acid	DHA:AA ratio
Sex				
Male	195	1.11 (0.89, 1.30)	6.15 (5.21, 7.25)	0.18 (0.15, 0.20)
Female	210	1.20 (0.99, 1.51)	6.25 (5.30, 7.16)	0.20 (0.16, 0.24)
P value[2]		0.0004	0.96	0.0001
Age group, y				
9.5 to <12	83	1.17 (0.93, 1.40)	6.41 (5.23, 7.08)	0.19 (0.16, 0.22)
12 to <14	123	1.15 (0.93, 1.44)	6.04 (5.20, 7.06)	0.19 (0.17, 0.22)
14 to <16	82	1.10 (0.98, 1.39)	6.17 (5.47, 7.02)	0.18 (0.16, 0.22)
16 to <18	117	1.17 (0.94, 1.36)	6.43 (5.21, 7.41)	0.19 (0.15, 0.22)
P trend		0.97	0.51	0.84
BMI *z*-score				
<0	43	1.17 (1.01, 1.48)	6.72 (5.42, 7.49)	0.18 (0.16, 0.22)
0 to <1	102	1.16 (0.95, 1.48)	6.35 (5.48, 7.29)	0.19 (0.16, 0.22)
1 to <2	102	1.18 (0.98, 1.37)	6.12 (5.23, 7.25)	0.19 (0.16, 0.23)
≥2	156	1.10 (0.89, 1.34)	5.91 (4.93, 7.03)	0.19 (0.16, 0.22)
P trend		0.02	0.01	0.36
Maternal education, y				
≤9 (secondary or primary)	155	1.13 (0.92, 1.33)	6.21 (5.27, 7.25)	0.18 (0.16, 0.21)
10 to <12 (some high school)	51	1.22 (0.92, 1.47)	6.10 (5.42, 7.09)	0.20 (0.17, 0.23)
12 (completed high school)	139	1.16 (0.97, 1.49)	6.44 (5.44, 7.38)	0.19 (0.16, 0.22)
>12	56	1.10 (0.93, 1.43)	5.64 (4.94, 6.91)	0.19 (0.17, 0.23)
P trend		0.18	0.82	0.66
Physical activity quartiles, h/wk				
Q1, 0 to <5.8	110	1.16 (0.96, 1.46)	6.11 (5.37, 7.02)	0.20 (0.16, 0.23)
Q2, 5.8 to <9.3	100	1.18 (0.94, 1.42)	6.14 (5.26, 7.29)	0.19 (0.16, 0.23)
Q3, 9.3 to <14.3	100	1.13 (0.98, 1.36)	6.33 (5.25, 7.31)	0.19 (0.15, 0.23)
Q4, 14.3 to 29	95	1.13 (0.89, 1.36)	6.37 (5.04, 7.25)	0.18 (0.16, 0.21)
P trend		0.30	0.55	0.78
Screen time quartiles, h/wk				
Q1, 1 to <23	104	1.14 (0.92, 1.48)	6.25 (5.21, 7.07)	0.19 (0.16, 0.23)
Q2, 23 to <33	107	1.19 (0.97, 1.40)	6.40 (5.48, 7.38)	0.19 (0.16, 0.22)
Q3, 33 to <48.5	96	1.17 (0.88, 1.41)	6.15 (5.21, 7.08)	0.19 (0.16, 0.22)
Q4, 48.5 to 116	98	1.10 (0.94, 1.36)	5.93 (5.19, 7.19)	0.18 (0.15, 0.22)
P trend		0.22	0.57	0.92
Ever smoked cigarettes				
No	317	1.16 (0.94, 1.43)	6.19 (5.23, 7.17)	0.19 (0.16, 0.22)
Yes	86	1.10 (0.96, 1.26)	6.20 (5.25, 7.25)	0.19 (0.16, 0.22)
P value		0.45	0.96	0.52
Caffeine intake quartiles, mg/d				
Q1, 0 to <0.10	102	1.14 (0.91, 1.36)	6.42 (5.25, 7.18)	0.18 (0.16, 0.21)
Q2, 0.10 to <0.63	104	1.19 (1.00, 1.44)	6.02 (5.31, 7.29)	0.19 (0.16, 0.23)
Q3, 0.63 to <33	98	1.12 (0.93, 1.41)	6.10 (5.21, 7.06)	0.19 (0.16, 0.22)
Q4, 33 to 681	101	1.14 (0.94, 1.42)	6.22 (5.11, 7.25)	0.19 (0.16, 0.23)
P trend		0.55	0.89	0.91
Canned tuna/sardine intake, g/d				
0	236	1.09 (0.89, 1.32)	6.22 (5.35, 7.18)	0.18 (0.15, 0.20)
>0 to <14	66	1.19 (1.04, 1.52)	6.11 (5.04, 6.98)	0.21 (0.18, 0.24)
14 to 251	103	1.24 (1.04, 1.54)	6.25 (4.99, 7.26)	0.21 (0.17, 0.25)
P trend		<0.0001	0.57	<0.0001

[1] Values are median (interquartile range). AA, arachidonic acid.
[2] For dichotomous characteristics, *P* values are from Wilcoxon tests. For ordinal characteristics, values of *P* for trends are from linear regression models with plasma fatty acid as the dependent variable and a continuous variable representing ordinal categories of the sociodemographic or lifestyle predictor as the independent variable.

20–30-min increase in sleep duration can be beneficial for children in terms of emotional control and academic outcomes (31, 32), the magnitudes of the reported associations are clinically meaningful and suggest a potential role of dietary PUFAs in promoting healthy sleep among adolescents.

Prior studies that have assessed DHA intake in relation to sleep in pediatric populations are scarce but in general corroborate a protective association between DHA intake and healthier sleep. Two studies in children with ADHD or school performance issues found better sleep quality and longer sleep duration in children in the experimental arm compared with

TABLE 2 Bivariate and minimally adjusted associations between plasma fatty acids and actigraphy-assessed weekday sleep characteristics in a sample of 405 Mexican youth[1]

	n	Weekday duration,[2] min		Weekday midpoint,[3] decimal h		Fragmentation index,[4] %	
DHA, % of total FA							
Q1 (median = 0.81)	102	498 (464, 541)[5]	10 (−11, 31)[6]	3.7 (2.7, 4.9)[5]	0.33 (−0.04, 0.70)[6]	11.6 (9.8, 13.6)[5]	0.8 (−0.2, 1.9)[6]
Q2 (median = 1.05)	101	515 (468, 581)	19 (−2, 40)	4.6 (3.1, 5.2)	0.82 (0.46, 1.19)	11.4 (9.2, 14.8)	0.8 (−0.3, 1.8)
Q3 (median = 1.25)	101	499 (447, 548)	−2 (−24, 19)	3.3 (2.6, 4.6)	0.07 (−0.30, 0.45)	11.8 (8.9, 14.5)	0.5 (−0.5, 1.6)
Q4 (median = 1.61)	101	501 (451, 551)	Reference	3.4 (2.5, 4.3)	Reference	10.9 (8.6, 13.7)	Reference
P trend[7]			0.12		0.003		0.11
AA, % of total FA							
Q1 (median = 4.6)	95	513 (467, 551)	Reference	4.0 (2.9, 5.1)	Reference	11.5 (9.4, 14.0)	Reference
Q2 (median = 5.8)	109	500 (460, 566)	−7 (−28, 13)	3.5 (2.7, 4.9)	−0.30 (−0.66, 0.07)	11.4 (9.3, 13.7)	−0.1 (−1.1, 0.9)
Q3 (median = 6.7)	104	509 (470, 563)	−2 (−23, 19)	3.7 (2.8, 4.7)	−0.22 (−0.59, 0.15)	11.8 (9.6, 14.6)	0.3 (−0.7, 1.4)
Q4 (median = 7.8)	97	498 (438, 535)	−20 (−41, 0)	3.3 (2.5, 4.7)	−0.51 (−0.88, −0.14)	10.9 (8.6, 14.7)	−0.2 (−1.3, 0.8)
P trend			0.09		0.01		0.83
DHA:AA ratio							
Q1 (median = 0.14)	102	500 (456, 551)	−1 (−22, 21)	3.7 (2.8, 4.9)	0.22 (−0.16, 0.59)	12.2 (9.8, 15.2)	0.6 (−0.4, 1.7)
Q2 (median = 0.17)	101	495 (446, 540)	−16 (−36, 5)	4.0 (2.6, 5.1)	0.21 (−0.16, 0.59)	10.9 (8.8, 13.5)	−0.4 (−1.4, 0.6)
Q3 (median = 0.20)	101	511 (452, 557)	−6 (−27, 15)	3.8 (2.7, 4.9)	0.21 (−0.17, 0.58)	11.5 (8.5, 14.0)	−0.1 (−1.2, 0.9)
Q4 (median = 0.25)	101	516 (467, 574)	Reference	3.4 (2.6, 4.7)	Reference	11.5 (9.4, 14.1)	Reference
P trend			0.77		0.28		0.29

[1] AA, arachidonic acid; FA, fatty acid; Q, quartile.
[2] Sleep duration averaged over the weekday nights (Sunday through Thursday).
[3] Sleep midpoint (median of sleep onset and wake time) averaged over the weekday nights.
[4] Calculated as the percentage of 1-min periods of sleep out of total number of sleep bouts of any length, averaged over the 7-d wear time.
[5] Values are median (interquartile range).
[6] Values are adjusted differences (95% CIs); from linear regression models with sleep characteristic as the dependent variable, indicator variables for quartiles of plasma fatty acid as the independent predictor; and sex, continuous age, and indicator variables for categories of maternal education and BMI-for-age *z*-scores as potential confounders. Plasma DHA and AA were run in separate models.
[7] Values of *P* for trends are from Wald tests in adjusted linear regression models with a continuous variable representing ordinal categories of plasma fatty acid quartiles.

the placebo (15, 17), although it is important to note that 1 of the studies (15) only found an association of DHA and sleep duration in a small subset of 43 children who had wrist actigraphy measurements. The importance of objective measurements of sleep could explain why a different study in Norwegian children (16) that utilized self-reported sleep failed to observe an association between fatty fish supplementation and sleep. In addition to objective measurements of sleep through wrist actigraphy, another distinguishing feature of the present study is the examination of sleep timing in addition to sleep duration. The timing of sleep is particularly relevant in our target population of adolescents, because puberty coincides with natural delays in circadian rhythms, which can make adolescents particularly susceptible to late bedtimes (33). Further, none of the prior studies examined weekdays compared with weekends separately, which could potentially

TABLE 3 Fully adjusted associations between plasma fatty acids and actigraphy-assessed weekday sleep characteristics in a sample of 405 Mexican youth[1]

	Weekend duration, min	*P* trend[2]	Weekday midpoint, h	*P* trend[2]	Fragmentation, adjusted difference	*P* trend[2]
DHA, % of total FA		0.005		0.07		0.09
Q1 (median = 0.81)	−32 (−57, −7)[3,*]		0.14 (−0.29, 0.58)[3]		1.0 (−0.2, 2.3)[3]	
Q2 (median = 1.05)	−21 (−44, 1)		0.75 (0.36, 1.14)*		0.8 (−0.3, 1.9)	
Q3 (median = 1.25)	−4 (−26, 17)		0.04 (−0.33, 0.42)		0.5 (−0.6, 1.6)	
Q4 (median = 1.61)	Reference		Reference		Reference	
AA, % of total FA		0.72		0.18		0.41
Q1 (median = 4.6)	2 (−22, 27)		0.36 (−0.07, 0.78)		−0.3 (−1.5, 0.9)	
Q2 (median = 5.8)	9 (−14, 31)		0.01 (−0.38, 0.40)		−0.3 (−1.4, 0.8)	
Q3 (median = 6.7)	1 (−21, 22)		0.13 (−0.24, 0.50)		0.4 (−0.7, 1.4)	
Q4 (median = 7.8)	Reference		Reference		Reference	
DHA:AA ratio		0.24		0.28		0.32
Q1 (median = 0.14)	−10 (−31, 12)		0.22 (−0.16, 0.60)		0.6 (−0.4, 1.7)	
Q2 (median = 0.17)	−11 (−32, 10)		0.24 (−0.14, 0.61)		−0.4 (−1.4, 0.6)	
Q3 (median = 0.20)	1 (−20, 22)		0.23 (−0.16, 0.60)		−0.1 (−1.1, 0.9)	
Q4 (median = 0.25)	Reference		Reference		Reference	

[1] AA, arachidonic acid; FA, fatty acid; Q, quartile. *Statistically significantly different from the reference at $P < 0.05$.
[2] Values of *P* for trends are from Wald tests in adjusted linear regression models with a continuous variable representing ordinal categories of plasma fatty acid quartiles.
[3] Values are adjusted differences (95% CI); from linear regression models with sleep characteristic as the dependent variable, indicator variables for quartiles of plasma DHA, plasma AA, caffeine intake, a continuous variable for total energy intake; as well as sex, age, and indicator variables for maternal education and BMI-for-age *z*-scores.

mask some associations that were only evident on weekends, when there is more flexibility in sleep schedules. Indeed, we found no associations with sleep duration during the weekday nights.

The observed association between lower DHA concentrations and delayed sleep timing could potentially be explained by the action of the hormone melatonin. Melatonin is a hormone produced in the pineal gland that is intricately involved in the sleep/wake cycle (34); the release of melatonin at night prepares the body for sleep, inducing drowsiness and the eventual onset of sleep. Melatonin release can be delayed by internal (e.g., pubertal stage) and external (e.g., light) stimuli. Animal studies have shown that lower intake of DHA is directly related to lower concentrations of DHA in the pineal gland of the brain as well as dysregulated melatonin release (5, 6). Our findings suggest that DHA intake could potentially hasten or intensify the melatonin release in humans as well. Future laboratory studies are needed to confirm whether DHA or fatty fish intake is associated with the timing of the dim-light melatonin onset and sleep timing in humans, since most previous studies examined sleep duration or quality (15–17) rather than timing. The source of DHA in our study population was primarily canned tuna and sardines; thus, we cannot rule out the possibility that the associations we observed were explained by other substances in these fatty fish. For example, vitamin D (35) as well as B vitamins have been related to sleep duration and quality in some studies (36).

Of note, the association between DHA and sleep timing was nonlinear, such that the largest difference in sleep timing was between the second-lowest DHA group (2nd quartile) and the highest DHA group (4th quartile). Compared to participants with the highest concentrations of DHA, participants with the lowest concentrations of DHA had a later sleep midpoint (as expected), but the difference was not statistically significant. Although these nonlinear results should be interpreted cautiously, a potential reason is that the source of DHA in this population, fatty fish, also contain toxicants such as mercury, which could hinder sleep (37). Thus, the group with the lowest exposure to fatty fish could not only have the lowest concentrations of DHA (possibly harmful) but also the lowest concentrations of mercury (possibly beneficial).

Interestingly, we found that the ratio of DHA:AA was not associated with sleep, because a higher AA was actually associated with an earlier sleep midpoint (albeit not statistically significant in fully adjusted models), in contrast to expectation. Nonetheless, some animal studies have pointed to a possible beneficial role of AA on sleep (38). The association also has biological plausibility, because AA is a precursor of anandamide, a cannabinoid that is involved in the initiation of sleep (39).

There are both strengths and limitations to consider in the present analysis. The major strengths include laboratory assessment of the fatty acids and an objective measurement of sleep via 7-d wrist actigraphy. One limitation is the fact that in contrast to plasma DHA, plasma concentrations of AA are not likely to be a valid proxy for intake of AA. Second, despite this being a relatively large study sample with objective measures, we might have been underpowered to detect smaller changes in sleep outcomes. Third, the generalizability of our findings could be limited to adolescents in Latin American countries. In addition, because the plasma DHA concentrations in this population were relatively low, the findings might not be generalizable across the entire range of DHA concentrations that have been observed in human plasma. We lacked information on the timing of daily activities, including the timing of last meal/snack or timing of screen use, a potential source of unmeasured confounding. Finally, although the blood collection preceded the sleep monitoring, we cannot negate the possibility that reverse causation could explain the findings (assuming the sleep measurements were a valid proxy for typical sleep patterns).

In summary, in a sample of Mexican peripubertal adolescents, we found that higher DHA concentrations were related to earlier sleep timing and longer sleep duration on the weekends. Further, the effect sizes were of clinical relevance; for example, there was a 45-min earlier sleep midpoint in the highest compared with the second-lowest DHA groups, and a 30-min longer sleep duration on the weekends in the highest compared with the lowest DHA groups. Whether DHA supplementation, as a part of a comprehensive sleep hygiene intervention (which includes maintaining consistent bedtime routines), could benefit sleep during the adolescent transitional period deserves investigation in randomized trials.

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